Histochemical Evaluation of Prostate Diseases: Integrating Bacterial Infections and Cancer Subtyping

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Abstract
Background: Prostate diseases, including bacterial infections and prostate cancer, pose significant health concerns worldwide. However, limited research has focused on the comprehensive characterization of bacterial infections in prostate diseases. This study aimed to address these knowledge gaps by investigating the prevalence of bacterial infections in prostate diseases as well as prostate cancer subtypes.

Methods: A retrospective study using formalin-fixed paraffin-embedded (FFPE) prostate biopsy tissue samples from patients diagnosed with prostate diseases. Relevant clinical information was collected, and Gram staining was performed to detect the presence of Gram-negative bacteria. Southgate's mucicarmine staining was utilized to categorize prostate cancer subtypes.

Data were analyzed using SPSS version 20.0.

Results: Among the 50 patients included in the study, the majority (48.0%) fell within the age range of 60-69 years. Prostatic hyperplasia was the most prevalent prostate disease (70.0%), followed by prostate cancer (28.0%). Gram-negative bacteria were identified in 56% of the FFPE. Categorization of prostate cancer subtypes using mucicarmine staining revealed that 71.4% of FFPE exhibited a positive reaction, indicating the presence of mucin.

Conclusion: This study has identified bacterial infections in prostate diseases, focusing on Gram-negative bacteria, mucin positive prostate cancer subtype using mucicarmine staining were as well identified.

Introduction
Prostate diseases, encompassing bacterial infections and prostate cancer, are prevalent health concerns affecting a significant number of individuals worldwide. Bacterial infections in the prostate can result in prostatitis, leading to symptoms such as pain, urinary difficulties, and compromised quality of life [1]. On the other hand, prostate cancer is one of the most common cancers among men, with diverse clinical presentations and outcomes [2]. While bacterial infections and prostate cancer are well-studied areas, limited research has been conducted on...
the comprehensive characterization of bacterial infections, particularly Gram-negative bacteria, in prostate diseases, and the categorization of prostate cancer into distinct subtypes [3]. Understanding the prevalence and impact of bacterial infections, as well as the heterogeneity of prostate cancer, is vital for improving diagnostic accuracy, treatment selection, and patient outcomes.

Recent studies have highlighted the importance of bacterial infections in prostate diseases. Nickel emphasized the current concepts and antimicrobial therapy for prostatitis, emphasizing the need for further research on bacterial characterization [1]. Additionally, Siegel et al. provided comprehensive cancer statistics, underscoring the significance of prostate cancer as a global health issue [2]. Nevertheless, limited research has focused on the specific characterization of bacterial infections and the categorization of prostate cancer subtypes.

To bridge this knowledge gap, the present study aims to comprehensively characterize bacterial infections in prostate diseases, with a specific focus on Gram-negative bacteria. Furthermore, we seek to establish a robust categorization system for prostate cancer subtypes.

Materials and Methods

Study Design: This retrospective study was conducted at the University of Maiduguri Teaching Hospital. Formalin-fixed paraffin-embedded (FFPE) prostate biopsy tissue samples from patients diagnosed with prostate diseases between January 2022 and December 2022 were included, while FFPE tissue samples with inadequate tissue content or poor preservation were excluded from the analysis. Samples with missing or incomplete clinical information were also excluded.

Sample collection and preparation:
FFPE Prostate Biopsy Tissue Retrieval: FFPE prostate biopsy tissue samples were retrieved from the hospital’s histopathology archive. A total of 50 samples met the inclusion criteria and were included in the study.

Sample Identification and Data Collection: Relevant clinical information, including patient demographics and pathological reports, was collected from patient records and anonymized for analysis.

Gram staining for bacterial detection:
FFPE tissue sections (3μm thick) were deparaffinized by sequential immersion in xylene and graded alcohols, the sections were rehydrated by rinsing in distilled water and then subjected to Gram staining technique following the laboratory’s protocol. The stained sections were observed under a light microscope at 100x and 400x magnifications, and the presence of Gram-negative bacteria was identified based on their characteristic morphology. The number of tissue sections positive for Gram-negative bacteria were then recorded and the frequency of Gram-negative bacteria in various prostate diseases was calculated and presented as percentages.

Categorization of prostate cancer subtypes:
FFPE tissue sections from prostate cancer cases were subjected to Southgate’s mucicarmine staining following the laboratory’s protocol to categorize prostate cancer subtypes into mucinous and non-mucinous. The stained sections were observed under a light microscope at 100x and 400x magnifications, and the presence of mucin was assessed based on the specific staining pattern.

Statistical Analysis:
Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. The frequency of Gram-negative bacteria in different prostate diseases and the categorization of prostate cancer subtypes were analyzed using appropriate SPSS statistical package version 20.0.

Ethical Considerations:
Given that the study utilizes formalin-fixed paraffin-embedded (FFPE) tissue samples, which are archival samples obtained during routine clinical procedures, and their use for research purposes does not involve direct patient contact or require ethical clearance. However, institutional guidelines and regulations regarding the use of FFPE tissue samples, ensuring that patient confidentiality and privacy are maintained throughout the study was adhered to.

Results

Table 1 presents the distribution of prostate diseases based on age groups. Among the 50 patients included in the study, the majority (48.0%) fell within the age range of 60-69 years, followed by 32.0% in the 50-59 age group. A smaller proportion of patients were observed in the age groups of 70-79 years (10.0%) and 80 years and above (10.0%).

Table 1: Distribution of Prostate Diseases Across Different Age Groups

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-59</td>
<td>16</td>
<td>32.0</td>
</tr>
<tr>
<td>60-69</td>
<td>24</td>
<td>48.0</td>
</tr>
<tr>
<td>70-79</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>80 and above</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 2 displays the frequency distribution of prostate diseases. Out of the total 50 cases analyzed, the majority (70.0%) were diagnosed with hyperplasia. Prostate Cancer accounted for 28.0% of the cases, while chronic nonspecific inflammation was observed in only 2.0% of the cases.

<table>
<thead>
<tr>
<th>Laboratory Diagnosis</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatic hyperplasia</td>
<td>35</td>
<td>70.0</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>14</td>
<td>28.0</td>
</tr>
<tr>
<td>Chronic Nonspecific Inflammation</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3 presents the correlation between different prostate diseases and Gram's reaction. Out of the total 50 cases analyzed, Gram positive bacteria were demonstrated in 22 cases (44%) while Gram negative bacteria were demonstrated in 28 cases (56%). Among the specific clinical conditions, hyperplasia had 14 cases (70%) with a positive Gram's reaction and 21 cases (70%) with a negative Gram's reaction. Adenocarcinoma showed 7 cases (28%) with a positive Gram's reaction and 7 cases (28%) with a negative Gram's reaction. Chronic nonspecific inflammation had only 1 case (2%) and showed positive Gram's reaction.

<table>
<thead>
<tr>
<th>Laboratory Diagnosis</th>
<th>Gram's Reaction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Prostatic hyperplasia</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Chronic Nonspecific Inflammation</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22 (44%)</td>
<td>28 (56%)</td>
</tr>
</tbody>
</table>

Table 4: The table displays the results of Southgate's mucicarmine reaction in prostate cancer cases. Out of the 14 total cases analyzed, 10 cases (71.4%) exhibited a positive mucicarmine reaction, indicating the presence of mucin. On the other hand, 4 cases (28.6%) showed a negative reaction.

<table>
<thead>
<tr>
<th>Southgate’s Mucicarmine Reaction</th>
<th>Prostate Cancer Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>10</td>
<td>71.4</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5 presents the distribution of prostate cancer cases based on age ranges. Out of the total 14 cases analyzed, the highest proportion (42.9%) of prostate cancer cases was observed in the age range of 50-59. The 60-69 age group accounted for 35.7% of the cases, followed by 14.3% in the 70-79 age group. The lowest proportion, 7.1%, was observed in the age group of 80 and above.

<table>
<thead>
<tr>
<th>Age range</th>
<th>Cancer cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 – 59</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td>60 – 69</td>
<td>5</td>
<td>35.7</td>
</tr>
<tr>
<td>70 – 79</td>
<td>2</td>
<td>14.3</td>
</tr>
<tr>
<td>80 and above</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion
The present study aimed to identify bacterial infections in prostate diseases and to categorize prostate cancer subtypes using Southgate's mucicarmine reaction in mucinous and non-mucinous.
Figure 2: Tissue Gram’s stain reaction, demonstrating gram positive and gram negative bacteria (positive control slide, bar = 50 microns)

Figure 3: Gram negative bacteria highlighted by Tissue Gram’s stain technique in prostate Adenocarcinoma. (bar = 50 microns)

Figure 4: Gram positive bacteria highlighted by Tissue Gram’s stain technique in prostate adenocarcinoma. (bar = 50 microns)

Figure 5: Haematoxylin and Eosin Stain, showing prostate adenocarcinoma (bar = 50 microns)

Figure 6: Mucin deposition highlighted by the Southgate’s Mucicarmine special stain in appendix tissue. Mucin appear pink brown to red colour. (positive control slide, bar = 50 microns)

Figure 7: Mucin deposition highlighted by the Southgate’s Mucicarmine special stain in Prostate adenocarcinoma. Prostatic glands and fibromuscular stroma all covered in mucin. Numerous mucin are seen (marked by arrow). (bar = 50 microns)
The results of this study demonstrated that the majority of patients fell within the age range of 60-69 years (48.0%), followed by the 50-59 age group (32.0%). This finding is consistent with previous studies that have identified older age as a significant risk factor for the development of prostate diseases as pointed by Etzioni et al., Siegel et al. This may suggest that risk of prostate cancer increases substantially after the age of 50 and continues to rise with advancing age [2,3]. Similarly, Anderson et al. found higher prevalence of prostatitis in men aged 50 years and above, this aligns with our study’s observation of a significant proportion of prostate diseases occurring in individuals within the 60-69 age range [4].

This study has further been supported by Yeboah who reported progressive increase in BPH prevalence with advancing age, with a higher proportion of cases observed in men aged 60 years and older thus supporting the notion that prostate diseases, including prostate cancer, prostatitis, and BPH, are more prevalent in older age groups [5].

Majority of cases (70.0%) in the study were diagnosed with prostatic hyperplasia. Prostatic hyperplasia or benign prostatic hyperplasia (BPH). This finding is consistent with previous research highlighting the high prevalence of prostatic hyperplasia in older men [6].

Prostate cancer accounted for 28.0% of the cases in the study. The observed frequency aligns with the global burden of prostate cancer, as it is one of the most commonly diagnosed cancers in men worldwide [7].

The association between prostate cancer and Gram's reaction suggests the potential involvement of bacterial factors in the development and/or progression of prostate cancer. Chronic nonspecific inflammation was observed in only 2.0% of the cases. Chronic inflammation of the prostate gland, even without a specific cause, has been implicated in the pathogenesis of various prostate diseases, including prostatic hyperplasia and prostate cancer [8,9]. The low frequency of chronic nonspecific inflammation in this study might indicate that other factors, such as bacterial infections or specific inflammatory processes, maybe associated with the observed prostate diseases.

Correlating prostate diseases and Gram's reaction showed that Gram-negative bacteria were more prevalent (56%). This finding is consistent with previous studies identifying Gram-negative bacteria as common pathogens in prostatic infections [1,10] implying the role for chronic bacterial prostatitis in the development and progression to prostatic hyperplasia and cancers.

Categorization of prostate cancer subtypes using Southgate's mucicarmine reaction revealed that 71.4% of the prostate cancer cases exhibited a positive reaction, indicating that they are mucinous. This finding is in line with Peter et al. reported the occurrence of mucinous subtypes of prostate cancer [12]. Only 28.6% of the prostate cancer cases are non-mucinous. Mucinous prostate cancer subtypes are characterized by the production of abundant extracellular mucin and are aggressive, high grade and poor prognosis compared to non-mucinous prostate cancer [13].

The age-specific distribution of prostate cancer cases has important implications for prostate cancer screening and early detection strategies. It underscores the importance of targeted screening efforts in the age groups with the highest incidence, such as men in their 50s and 60s. Furthermore, it emphasizes the need for individualized approaches to prostate cancer management, considering factors such as age, overall health status, and life expectancy.

**Conclusion**

In conclusion, this study provides valuable insights in the involvement bacterial infections in prostate diseases, and categorization of prostate cancer subtypes.

**Limitation**

The retrospective nature of the study and the reliance on FFPE tissue samples may have introduced selection bias. Additionally, the relatively small sample size limits the generalizability of the findings.

**Conflict of Interest**

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

**References**


